TISSUE CULTURE AND PROTOPLASTS OF TWO RARE ARACEAE (AROID) PLANTS

BY

HAEI DONG (PHILIP) SHAW

A THESIS SUBMITTED FOR THE DEGREE OF
MASTER OF SCIENCE

FACULTY OF SCIENCE
DEPARTMENT OF ENVIRONMENTAL SCIENCES
UNIVERSITY OF TECHNOLOGY, SYDNEY
AUSTRALIA

1999

CERTIFICATE

I certify that this thesis has not already been submitted for any degree, and is not being submitted as part of candidature for any other degree.

I also certify that the thesis has been written by me and that any help that I have received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

Signature of Candidate

HAEI DONG (PHILIP) SHAW

ACKNOWLEDGMENTS

I firstly wish to thank my project supervisor, Dr. Lou De Filippis for his review of my English in writing this thesis, and for his continued guidance and instructions during the course of this project.

I further wish to extend my thanks to Dr. Alistair Hay, Senior Horticulturist of Royal Botanic Gardens Sydney, for his invaluable assistance in providing experimental plant material, and for his comments and support during this project.

I am indebted to Ms. Belinda Kennedy for assistance in the collection of literatures and her encouragement.

For invaluable advice in this field, I would like to thank Mr. David Chen, he was not only a fellow in the laboratory, but my best brother in Christ.

I would also like to thank Dr. Brett Summerell, Plant Pathologist at the Royal Botanic Gardens Sydney, in making available facilities, including laboratory space during the initiation of some of the experiments.

Thanks also go to Dr. Krystyna Johnson for some critical discussion and supervision while my supervisor was on study leave.

Last, but not the least I would like to thank my wife Tjih-Ian (Science) for reviewing the manuscript, and for her continued encouragement and support for the duration of the project.

SUMMARY

As a major family of tropical rainforest plants, *Araceae* or Aroids are not only of horticultural importance, but also have some agricultural significance in the eastern tropics and subtropics. On the other hand, many species are rare and endangered, and the need for conserving them from extinction is urgent. However, details of their propagation in general is hampered by the slow rate of vegetative multiplication. This project therefore describes studies on the *in vitro* micropropagation of *Alocasia lauterbachiana* Engler and *Homalomena davidiana* A. Hay, as a beginning to more detailed studies on the propagation of Aroid in general.

Protoplasts were successfully isolated. The best digestion enzyme combination was 2% cellulysin (from *Trichoderma viride*), plus 1% cellulase (from *Aspergillus niger*) and 1% cellulase (from *Penicillium funiculosum*). A period of 4-5 hours of incubation time produced a maximum number of isolated protoplasts at 25^0C.

A successful protocol for the rapid micropropagation of both Aroid species was developed, which combined callus induction and a high rate of shoot multiplication, followed by root development. Plantlets could then be directly transferred to green house acclimatization conditions, with high survival rates.

The effects of different explant sources, various auxins and cytokinins, and basal media on callus and shoot initiation were determined. Half strength Murashige and Skoog media was superior to full strength and quarter strength media. The best plant growth regulator and concentration was 5.0 μM thidiazuron for continued callus induction, and 2.5 μM thidiazuron for continued callus growth. Root development was best achieved by 0.25 μM naphthalene acetic acid.

The present study therefore introduced a tissue culture method aimed at preserving cell lines and tissues derived from differentiated callus and shoots, which should aid in the preservation of these two rare Aroid species; of which one (*Alocasia lauterbachiana*) is also endangered.

CONTENTS

ABBREVIATIONS

BA(BAP)	benzyladenine: benzylaminopurine
BME	β-mercapto-ethanol
BSA	bovine serum albumen
2,4-D	2,4-dichlorophenoxyacetic acid
FW	fresh weight
g	gram
GA	gibberellic acid
IAA	1H-indole-3-acetic acid
IBA	indole-3-butyric acid
K	kinetin; N^6 - furfuryl adenine
kPa	kilopascal
NAA	α-naphthaleneacetic acid
MES	2-(N-morpholino) ethanesulfonic acid
MS	Murashige and Skoog
MW	molecular weight
PAR	photosynthetic active radiation
PGR	plant growth regulators
PVP	polyvinyl-pyrrolidone
RPM	revolutions per minute
SE	standard error
SEM	scanning electron microscopy
TC	tissue culture
TEM	transmission electron microscopy
TDZ	thidiazuron
UV	ultraviolet light
WPM	woody plant medium
V/V	persent "volume in volume"
W/V	persent "weight in volume"
Z	zeatin; 2-mothyl-4-(1H-purin-6-ylamino)-2-buten-1-01

LIST OF FIGURES

LIST OF TABLES